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The enhancement of growth parameters in common carp (*Cyprinus carpio*) larvae using probiotic in rearing tanks and feeding by various *Artemia* nauplii

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Abstract. In probiotic trials the blend of *Bacillus circulans* and *Bacillus licheniformis* were added in rearing tanks of common carp at a concentration of 1×10^3 CFU mL^{-1}. *Artemia urmiana* (Gunther, 1899), *Artemia franciscana* and *Artemia parthenogenetica* nauplii were administered as live food to common carp larvae. Within treatments, final body weight (FBW), specific growth rate (SGR), thermal growth coefficient (TGC), daily growth coefficient (DGC), relative gain rate (RGR) were affected by probiotic bacillus ($P<0.05$). These growth and feeding parameters of protein efficiency ratio (PER), lipid efficiency ratio (LER) and energy efficiency ratio (EER) significantly increased in treatment of G.P-bacteria and G.F-bacteria where the common carp larvae fed on with *A. franciscana* and *A. parthenogenetica*. The highest growth and feeding parameters were obtained in treatment of G.P-bacteria while in treatment of G.U-bacteria were significantly ($P<0.05$) lower than other treatments. The best feeding performance of common carp larvae was obtained by feeding of *A. parthenogenetica* nauplii in trial of probiotic (G.P-bacteria).
Key Words: *Artemia*, probiotic, feeding performance, nauplii, growth efficiency.

Introduction. Probiotics can be defined as live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance (Fuller 1992). Successful colonization in digestive system of larvae involves competition with the established micro flora for attachment sites and nutrients. The species composition of the intestinal micro flora of fish larvae can be influenced at an early stage of development, when few, if any, bacteria are present in the larval gut, by addition of specific bacterial strains to the live food or the water (Ringø et al 1996). The probiotic bacteria can increase the digestive enzymes activity, digestibility of the ingested nutrients and enhancement of growth and feeding performance in fish larvae.

The use of probiotic bacillus has been suggested as an important strategy to accomplish reproducible outputs through biocontrol in cultivation systems for marine fish larvae and crustaceans (Nogami & Maeda 1992; Dragos et al 2010). The bacterial flora in the larval gut originates from the bacteria associated with the eggs, the water in the rearing tanks, and the live food (Olafsen & Hansen 1992). The gut of marine fish larvae is rapidly colonized by bacteria during the first days after hatching. Members of this pioneer community that colonize the gut at an early stage may acquire a competitive advantage compared with bacteria introduced at a later stage (Hansen & Olafsen 1999).

The brine shrimp *Artemia* spp. are common live food organisms used for the rearing of fish larvae. Growth responses of fish to brine shrimp will not only depend on the species but also on the geographical strains of *Artemia*. However, determination of feeding rate of live food such as *Artemia* and feeding efficiency of fish larvae is very important because the amount of *Artemia* nauplii fed to the cultured larvae will vary according to species of fish, density and developmental stage of fish larvae (Hertrampf & Piedad-Pascul 2000).

Among probionts, *Bacillus* are gram positive, spore forming bacteria, used commercially as probiotics; since the physical and biological characteristics of the spore, *Bacillus* preparations are resistant and have a long lasting shelf life and so they can be stored inventively in a desiccated form; moreover the production cost of spores for aquaculture is very convenient (Wang et al 2008). *Bacillus* can act positively on cultured organisms by enhancing survival and growth (Gomez-Gil et al 2000), by stimulating the digestive (Ziaei-Nejad et al 2006) and immune systems (Gatesoupe 1999) and by improving water quality in terms of bioremediation (Kennedy et al 1998). Most of these studies have aimed at improving the feeding utilization for growth by using different live food sources such as *Artemia* nauplii. There is no doubt that *Artemia* nauplii can be a good live food and energy sources and thereby improve the efficiency of growth in common carp larvae. These findings will be a major importance for the economical and nutritional sustainable development of commercial larviculture of this fish.

The objective of the present study was to investigate the ability of common carp larvae in exploitation of *A. urmiana*, *A. franciscana* and *A. parthenogenetica* nauplii and growth parameters in different feeding treatments in comparison with effect of addition of *B. circulans* and *B. licheniformis* to the culture medium or cultivation system of common carp (*Cyprinus carpio* Linnaeus, 1758) larvae.

Material and Method. Bacterial suspension. *B. circulans* and *B. licheniformis* (Protexin Aquatech, UK) were used in this study. The spores of *B. circulans* and *B. licheniformis* were rehydrated to vegetative bacteria according to manufacturer's instructions and the suspension of bacteria was used in concentration of 1×10^3 CFU/mL.

Hatching of cyst. *A. urmiana* cysts were donated by the *Artemia* and Aquatic Animals Research Institute of Urmia University. The *A. parthenogenetica* had been collected from the Urmia Lake and Lake Maharloo, and *A. franciscana* cysts were provided by the commercial companies. The chorion of the cysts was chemically removed by a process known as decapsulation, according to the methodology of Sorgeloos et al (1977).

Decapsulated cysts of *A. urmiana*, *A. franciscana* and *A. parthenogenetica* were incubated for 24 h in hatching tanks with a conical bottom in a standard condition of temperature 30°C, salinity of 30 ppt, oxygenated using electrical air pump and relatively of constant illumination (2000 Lux) on the water surface (Gomez-Gil et al 1998).

After 24 h, the newly hatched nauplii were collected aseptically on a 120 mm-pore-size sieve and washed thoroughly with distilled water. Proximate composition of the *A. urmiana*, *A. franciscana* and *A. parthenogenetica* carcass were analyzed. Moisture was determined by oven drying the weighed fresh sample at 100°C for 24 h; crude protein (nitrogen × 6.25) by micro-Kjeldahl digestion and distillation after acid digestion using a Kjeltec 1026 Distillation Unit together with a Tecator Digestion System (Tecator, Sweden); lipid was determined by extracting the residue with 40-60°C petroleum ether for 7-8 h in a Soxhlet apparatus and ash was determined by ignition at 550°C in a muffle furnace to constant weight.

Culture system of fish larvae. Twenty-day old common carp larvae with average weight of 120±10 mg were obtained from Woshmgir Fish Hatchery, Golestan, Iran. Fish larvae were acclimatized to laboratory condition for 5 days and fed with different *Artemia* nauplii. Each experimental tank was supplied with non-chlorinated water from a deep tube well with continuous aeration. The fish were transferred and randomly allocated at 40 fish per tank to 24 circular fiberglass tanks (capacity of 10 L).

In treatments G.P, G.F and G.U the common carp larvae were fed with *A. parthenogenetica*, *A. franciscana* and *A. urmiana* nauplii respectively and in other treatments (G.P-bacteria, G.F-bacteria and G.U-bacteria) the fish larvae were fed with these *Artemia* nauplii and the blend of two probiotic bacteria (*B. circulans* and *B. licheniformis*) were then added to the rearing tanks. This experiment was conducted in six trials with six treatments, each with four replicates.

The tanks were aerated to keep the live food in suspension, and illuminated by fluorescent tubes (40 W), water temperature was 24-26 °C, and the replacement of water was four times daily. Dead larvae and excessive food were removed daily from the tanks.

In trials of probiotic, the blend of *B. circulans* and *B. licheniformis* were added directly to the water of the tanks at a concentration of 1×10^3 CFU/mL at four times a day. The newly hatched nauplii were separated, rinsed and added to the fish tanks four times per day. The feeding rate was 30% of wet body weighs per day (Jafaryan et al 2009a). The period of experiment was 28 days. Samples of water from each tank were collected every day. Serial dilutions of the samples in distilled water and 2.0% (weight/volume) NaCl were plated on Tryptic Soy agar (TSA) and incubated at 30 °C for 24 h. After 24 h the colony forming units (CFU) was counted. At the end of the experiment all the fish were sampled and some growth and feeding parameters of fish larvae were calculated by following formula:

Daily growth coefficient (DGC) = 100× [(final body weight$^{0.333}$- initial body weight$^{0.333}$)/ days of experiment]. (De Silva & Anderson 1995)

Thermal growth coefficient (TGC) = [g final body weight$^{0.333}$-g initial body weight$^{0.333}$]/ [Water temperature× days of experiment] (Cho 1992).

Specific growth rate (SGR) = 100× [ln final weight of fish-ln initial weight of fish)]/days of feeding (Cho 1992).

Condition factor (CF) = 100× [(g final weight of fish)/ (total length of fish- cm)^3] (De Silva & Anderson 1995).

Average daily growth (ADG) =100× [(final weight of fish- initial weight of fish)/ (initial weight of fish).days of feeding] (De Silva & Anderson 1995).

Relative gain rate (RGR %) = 100× [(final weight of fish- initial weight of fish)/ initial weight of fish)] (De Silva & Anderson 1995).

Statistical analysis. Statistical analysis of data was performed by analysis of variance (ANOVA) using SPSS-17 followed by Duncan's multiple range tests.

Results and Discussion. As indicated in Table 1, the proximate compositions of the three studied *Artemia* were different. *A. urmiana* nauplii had the maximum crude protein (56.83%) while the lowest level of carcass crude protein (39.09%) was obtained in *A. parthenogenetica*.

Table1

Proximate carcass composition of the three experimental studied *Artemia* nauplii

Artemia \ composition	crude protein %	crude lipid %	crude energy (kcal/g)	dry matter %	moisture %	ash %
A. franciscana	40.65± 3.16	18.91± 4.2	4673± 351	11.76± 2.82	88.24± 5.66	10.09±1.2
A. urmiana	56.83±6.33	21.2±3.22	4727±245.6	9.09±1.22	90.91±6.25	3.75±0.20
A. parthenogenetica	39.09±2.33	17.86±1.02	4592±336	11.76±1.12	88.24±6.77	9.53±0.45

The crude lipid of *A. urmiana* was in highest level (21.20%) while in *A. franciscana* and *A. parthenogenetica* were 18.91% and 17.86%, respectively. Crude energy level in *A. franciscana* nauplii was 4673 kcal g^{-1} while in *A. parthenogenetica* and *A. urmiana* nauplii were 4592 and 4727 kcal g^{-1}, respectively. The values of growth parameters of common carp larvae in different treatments are presented in Table 2. The growth parameters were significantly affected by addition of probiotics in the culture media ($p<0.05$). The probiotic *Bacillus* had negative effect on growth parameters in the treatments G.U-bacteria.

The maximum final body weight (591.6 mg), final body length (37.87 mm), specific growth rate (5.65% body weight/day), thermal growth coefficient (0.472), daily growth coefficient (1.23), relative gain rate (493.2%) and average daily growth rate (17.60%) were observed in treatment C.P-bacteria and this was significantly different compared to other experimental treatments ($p<0.05$); while the highest condition factor (1.14) was obtained in treatment C.U-bacteria.

Table 2

The values of growth parameters of common carp larvae in different treatments

Treatment	C.P-bact.	C.F-bact.	C.U-bact.	C.P	C.F	C.U
I.B.W (mg)	120±10	120±10	120±10	120±10	120±10	120±10
F.B.W (mg)	591.6±94.2^{a}	562.8±101.3^{a}	498.2±88.4^{b}	493.4±58.4^{b}	468.9±98.1^{b}	389.2±73.9^{c}
F.B.L (mm)	37.87±2.16^{a}	36.69±3.59^{ab}	35.90±3.29^{b}	35.52±2.94^{bc}	34.48±3.27^{c}	32.34±2.59^{d}
SGR	5.65±0.59^{a}	5.36±0.92^{a}	4.96±0.80^{b}	4.94±0.68^{b}	4.73±0.95^{b}	4.09±0.78^{c}
TGC	0.472±0.062^{a}	0.445±0.110^{a}	0.403±0.086^{b}	0.400±0.088^{b}	0.380±0.07^{b}	0.319±0.08^{c}
DGC	1.23±0.169^{a}	1.16±0.22^{a}	1.05±0.20^{b}	1.04±0.22^{b}	0.99±0.18^{b}	0.82±0.151^{c}
RGR	493.0 ±78.5^{a}	368.9±80^{c}	315.1±59^{d}	411.1±76^{b}	290.8±72^{d}	224.3±65^{e}
CF	1.09±0.08^{ab}	1.12±0.17^{ab}	1.07±0.18^{b}	1.08±0.107^{ab}	1.12±11^{ab}	1.14±0.20^{a}
ADG	17.60±2.80^{a}	13.18±2.80^{c}	11.26±2.42^{d}	14.68±2.58^{b}	10.38±1.81^{d}	8.01±1.50^{e}

The different results of feeding parameters as PER, LER, EER and FCR between common carp larvae in different trials was a clear demonstration that the efficiency ratio of protein, lipid and energy of fish larvae were significantly ($p<0.05$) accreted in common carp larvae that fed on by *A. franciscana* and *A. parthenogenetica* with adding probiotic bateria.

The proximate carcass composition of common carp larvae in different treatments was showed in Table 3. The results indicated that the using probiotic in rearing tanks of common carp larvae significantly ($p<0.05$) promoted levels of dry matter in treatments of C.P-bacteria and C.U-bacteria in comparison with other treatments. The adding of probiotic bacillus in rearing tanks increased the crude lipid in treatment of C.P-bacteria in comparison with treatments of C.F-bacteria, C.P, C.F and C.U but did not have significant difference with treatment of C.P. The best level of crude energy (4608 cal/g) obtained in treatment of C.P-bacteria and had significant difference with other treatments ($p<0.05$).

Table 3

Proximate carcass composition of common carp larvae in different treatments

Treatments \ composition	C.P-bacteria	C.F-bacteria	C.U-bacteria	C.P-bact.	C.F-bact.	C.U-bact.
Dry matter (%)	30.5± 4.18^{a}	22.14± 2.1^{b}	29.44± 4.17^{a}	14.40± 1.3^{c}	18.64± 2.6^{b}	26.7±4.2^{b}
Crude protein (%)	68.62±5.45^{b}	71.24±8.22^{a}	70.53±4.32^{a}	67.7±1.35^{b}	70.85±1.0^{a}	71.6±2.3^{a}
Crude lipid (%)	12.78±2.45^{a}	11.08±1.05^{b}	11.32±2.45^{b}	12.05±1.4^{a}	11.16±1.8^{b}	10.7±1.5^{c}
Crude energy (cal/g)	4608±50^{a}	4423±45^{c}	4536±62^{b}	4561±46^{b}	4421±55^{c}	4407±46^{c}

This suggests that the addition of probiotics optimized the feed consumption and promoted growth parameters (Lara-Flores et al 2003). In the present investigation, dry matter could be significantly enhanced in common carp with adding probiotic bacillus in rearing tanks. Also adding the blend of *B. circulans* and *B. licheniformis* increased the crude lipid and crude energy in treatments of C.P-bacteria. In consent with our findings in the present study, the common carp (*C. carpio*) had best growth and feeding efficiency in feeding of *A. parthenogenetica* in comparison with *A. franciscana* and *A. urmiana* nauplii (Jafaryan et al 2009b). In contradiction with our results Boyd et al (1984) reported that the adding commercial probiotic bacteria didn't have any significant effect on growth parameters of channel catfish. Addition of bacteria bioencapsulated in *Artemia* metanauplii to a rearing system for halibut larvae (*Hippoglossus hippoglossus* Linnaeus, 1758) didn't increased the growth parameters of this fish (Makridis et al 2001).

The results of this study clearly demonstrate that the common carp larvae had different ability in exploitation of various *Artemia* nauplii and also addition of *B. circulans* and *B. licheniformis* to the culturing tank increased the growth rate of common carp larvae in the treatments of G.P-bacteria and G.F-bacteria. In trials of G.P, G.F and G.U where the common carp larvae fed on *A. parthenogenetica*, *A. franciscana* and *A. urmiana*, the best growth and feeding performance were obtained in treatment of G.P. while the fish larvae in treatment of G.U showed the lower ability of exploitation of *A. urmiana.* In concurrence with our findings in the present study, the Beluga (*Huso huso* Linnaeus, 1758) had best growth and feeding efficiency in feeding of bioencapsulated *A. urmiana* nauplii with probiotic bacillus (Jafaryan et al 2007b).

Administration of the *B. circulans* and *B. licheniformis* via direct inoculation to rearing tanks resulted significant higher growth and feeding performance in comparison with other treatments. The growth parameters of common carp larvae as final body weight (F.B.W), final body length (F.B.L), SGR, TGC, DGC, RGR, CF and ADG were significantly promoted in the treatments of G.P-bacteria and G.F-bacteria. Similar results were observed by Gatesoupe (1999) using *Bacillus toyoi* in turbot (*Scophthalmus maximus*), and by Swain et al (1996) in Indian carps, in which improved growth factors and feeding efficiency were recorded. In accordance with our findings in this study, using probiotic bacillus in *Artemia urmiana* nauplii broth, for feeding *Acipenser nudiventris* (Lovetsky, 1828) larvae had good effects on growth parameters (Jafaryan et al 2010). This is in agreement with the findings by Jafaryan et al (2007a) who also reported higher F.B.W, F.B.L, SGR and TGC for Persian sturgeon (*Acipenser persicus*) larvae. The relative gain rate (RGR) and average daily growth (ADG) observed for common carp larvae are similar to reported values for three species of Caspian sturgeon (*A. nudiventris*, *Acipenser persicus* (Borodin, 1897) and *H. huso*) larvae (Jafaryan et al 2010) in feeding of bioenriched *Artemia* nauplii with probiotic bacteria. The growth paramerts observed for common carp larvae are similar to reported values for silver carp (*Hypophthalmichthys molitrix*) larvae (Jafaryan et al 2009a) and Tillner et al (2009) for feeding of carp larvae (*Cyprinus carpio*) by *Artemia salina* nauplii.

The best exploitation of *Artemia* was obtained in treatment G.P-bacteria (addition of 1×10^6 CFU/L in rearing tanks) where the common carp larvae were fed with *A. parthenogenetica*. Nevertheless, the probiotics had negative effects on growth parameters of the common carp larvae in treatments G.U-bacteria in which the fish was fed with *A. urmiana* nauplii with probiotic bacteria in the rearing tanks. In confirmation of this result, Ghosh et al (2003) highlighted that using *B. circulans* in concentration of 1.5×10^4 CFU/g of diet, decreased the growth and feeding performance of rohu (*Labeo rohita* Hamilton, 1758) larvae. The similar effect was obtained by Jafaryan et al (2007b) using probiotic bacilli in concentration of 3×10^8 CFU/L in broth of *Artemia* nauplii for feeding of beluga (*H. huso*) larvae. Ghosh et al (2002) indicated the over concentration of probiotic bacillus reduced the growth parameters in rohu (*Labeo rohita*) larvae. They emphasized that the high specific activity of bacterial exteracllular enzymes had a negative effect on growth and feeding performance. This suggested that lower concentration of probiotic bacillus is necessary in rearing tank of common carp larvae to give predictable results in feeding by *A. urmiana* nauplii.

In agreement with these results Ziaei-Nejad et al (2006) reported that when the probiotic bacilli were added to rearing tanks at the concentration of 7.3×10^6 CFU/mL, increased the growth parameters of Indian white shrimp (*Fenneropenaeus indicus* Milne-Edwards, 1837) in comparison with control.

These particular bacterial probiotic had considerable extracellular amylolytic, cellulolytic, proteolytic and lipolytic activities (Bairagi et al 2002a) and is reported as reducing the antinuritional factors as tannins, phytates and mimosine to minimal values, reducing food conversion ratio (Bairagi et al 2002b) and as increasing growth performance in cultivable fish larvae (Bairagi et al 2004). In particular, *B. circulans* is known to produce proteases and other enzymes that enable it to contribute to the natural digestion activity of the host (Ziaei-Nejad et al 2006) and it can be a source of micro and macro-elements as feed (Verschuere et al 2000).

Furthermore, adding *B. circulans* and *B. licheniformis* in a concentration of 1×10^3 CFU/mL to the culturing system water promoted the consumption of *Artemia parthenogenetica* and *A. franciscana* nauplii by common carp larvae. This confirmed the results obtained in the studies of Carnevali et al (2004), in which *Lactobacillus fructivorans* and *Lactobacillus plantarum* were used for bioencapsulation of *A. franciscana* nauplii in feeding of sea bream (*Sparus aurata* Linnaeus, 1758) larvae. The results were also in accordance with the findings of Rengpipat et al (1998), in which the probiotic bacteria (*Bacillus* S11) were added in the culture medium of black tiger shrimp *Penaeus monodon (*Fabricius, 1798) and was proven by Gatesoupe (1999) in using *Bacillus toyoi* on turbot (*Scophthalmus maximus* Linnaeus, 1758) larvae. Commonly present in fish microflora (Sugita et al 1998), *B. licheniformis* has been shown to act as promoter. It has been found to produce extracellular proteases including amylase and cellulase, key enzymes involved in rohu fingerlings digestive activity (Ghosh et al 2002). Interestingly, *B. circulans* has been previously administered with other strains such as *B. licheniformis* in rainbow trout, leading to higher growth and immune resistance (Raida et al 2003; Bagheri et al 2008).

Conclusions. The present results indicated that the addition of probiotic bacilli to rearing tanks had different effects on the growth parameters of common carp larvae when they were fed on different *Artemia* nauplii.

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